REMARKS

Reconsideration of the above-identified application in view of the remarks following is respectfully requested.

Claims 1-14 are in this case. Claims 1-4 were withdrawn under a restriction requirement as drawn to a non-elected invention. Claims 5-14 have been rejected.

35 U.S.C. § 103(a) Rejections

The Examiner has rejected claims 5-14 under 35 U.S.C. § 103(a) as being unpatentable over Altman et al. (Proc. Nat. Acad. Sci. USA; 1993, 90: 10330-10334) in view of Matsumura et al. [J. Biol. Chem.; 1992, 267 (33): 23589-23595].

The Examiner points out that Altman teaches the production of soluble functional MHC class II complexes in E. coli following the purification of MHC class II from inclusion bodies and in vitro folding of the MHC molecules. In addition, the Examiner states that Altman teaches the association of the MHC molecules with antigenic peptides and that: (i) no other proteins are required for the efficient folding of the MHC molecules and (ii) carbohydrate modification is not necessary for T cell recognition.

Applicant agrees with the Examiner that Altman describes the reconstitution of class II MHC-peptide complexes from denatured subunits. In the Altman study the α and β subunits were synthesized as inclusion bodies in two separate *E. coli* transformants (Ec-I-E^k α and β); following expression, the subunits were re-folded *in vitro* in the presence of an antigenic peptide to form a complex which was stable only at a restricted range of pH conditions, *i.e.*, pH 7.4 - 7.6, and at limited temperatures (*i.e.*, 15 to 25 °C) and was completely unstable at physiological temperatures of 37 °C (see Figure 1b in Altman et al., 1993). Since the MHC complex formed in the Altman

study is unstable at physiological temperatures it is not functional, *i.e.*, it cannot replace the MHC complex formed *in vivo* (*i.e.*, the complex formed in the body of an organism at physiological temperatures).

In addition, Altman states that class I MHC proteins produced in eukaryotic expression vectors are unstable at physiological conditions (Altman et al., 1993, p. 10334, left column, lines 11-14). Further support for this statement can be found in Ljunggren HG et al., 1990; Nature (London) 346: 476-480.

With respect to Matsumura et al., the Examiner states that this reference teaches expression of soluble non-peptide bound MHC class I molecules in Drosophila melanogaster cells and illustrates *in vitro* binding of synthesized peptides to the MHC molecules. It should be noted that it is well known and widely accepted that a class I MHC complex cannot be correctly folded and thus purified in the absence of a peptide. In fact, as is described by Paul WE (Ed.) in 1999 (Fundamental Immunology, Forth edition; Lippincott-Raven publishers, pp. 273) "MHC-I biosynthesic pathway is dependent on proper generation of cytosolic peptides by the proteosome and delivery to the ER by TAP, appropriate core glycosylation in the ER, transport through the Golgi, and arrival at the plasma membrane".

Matsumura generated a truncated H-2K^b cDNA encoding the $\alpha 1 \alpha 2 \alpha 3$ extracellular domains and a full-length murine $\beta 2$m cDNA and co-transfected these two separate clones into S2/M3 Drosophila melanogaster cells. The expressed recombinant proteins were collected from the culture supernatant and purified using an anti K^b affinity column and ion exchange chromatography. However, due to the extremely low amounts of soluble complexes, Matsumura used immunoprecipitation with the Y-3 antibody to visualize the recombinant class I complex. In addition, as is shown in Figures 1c and d in Matsumura et al., in the presence of 1 % Triton detergent

(which was used to release the MHC complex from the aggregates) the purified class I MHC complex was completely unstable at physiological temperatures (*i.e.*, 37 °C). These results therefore demonstrate that Matsumura et al was unable to form a functional class I MHC complex in the eukaryotic system.

In sharp contrast to the references cited by the Examiner, the present invention relates to expressing in bacteria DNA construct encoding a single chain polypeptide in which the extracellular domain of HLA-A2 is attached to β-2 microglobulin via a 15-amino acid flexible linker [$(Gly\text{-}Ser_4)_3$]. This unique configuration is defined in claim 5 of the present invention which states: "a functional mammalian β-2 microglobulin amino acid sequence covalently linked to a functional mammalian MHC class I heavy chain amino acid sequence". Such a configuration is neither taught nor suggested by Altman and Matsumura. In addition, Applicant also strongly believes that the combination of the teachings of Altman and Matsumura would not motivate one of skill in the art to make the present invention. If such a motivation was indeed provided by their combined teachings as argued by the Examiner, then it stands to reason that in the time span that separates the date of these publications and the time of filing of the instant application (almost 10 years), one of ordinary skill in the art would have generated and published such configuration, and yet not a single publication proposing such unique configuration exists.

It appears as if since the time of Altman's publication (1993) all attempts to express MHC complexes in bacteria concentrated on dual expression systems and *in-vitro* refolding and not on fused expression of the two subunits. The fact that such a unique configuration was first suggested by the present inventor proves that the

teachings of Altman and Matsumura did not motivate the ordinary skilled artisan to pursue such a novel and unexpectedly successful approach.

The present inventor has shown that expression of such a DNA construct in E. coli surprisingly results in efficient production of a functional, and stable single chain MHC class I molecule which can be correctly folded in the presence of a specific HLA-A2-restricted peptide. The correctly folded peptide-MHC complexes of the present invention were monomeric, very pure, and functional. In fact, the yield of the refolded purified scMHC complex was 20-25 mg per 100 mg of refolded inclusion bodies protein, which is equivalent to 20-25 mg per liter of culture. This reflects 50 times more yield than that achieved by Altman et al. [200 μg of purified complex in every 500 ml reaction mixture (Altman et al., 1993, p. 103333, left column, lines 48-49)] in which expression was from two separated expression constructs encoding the α and β subunits of the class II MHC complex in E. coli. Aside from being highly pure, the scMHC complexes of the present invention were also extremely stable at physiological temperatures making such complexes highly suitable for various applications (e.g., studying disease-related immune responses, characterizing MHC-T-cell receptor (TCR) interactions), applications which cannot be carried out with the prior art complexes of Altman et al., 1993 or Matsumura et al., 1992. For example, analysis of the MHC complex melting curve showed that the complex containing peptide G9-209-2M was thermally stable with a melting temperature of approximately 60 °C (see Example 1 of the Examples section in the present invention).

These characteristics of the present complex, and in particular its ability to remain folded at temperatures as high as 60 °C, result from the covalent linkage between the β2 microglobulin and the HLA-A2 MHC molecule, a feature which can only be

attributed to the molecules of the present invention. Moreover, as is shown in Example 1, Figures 4 and 7-10 of the instant application, the class I MHC complex of the instant invention is identical in its structural and functional properties to the native complex.

The Examiner further states that what has been performed with class II MHC molecules in *E. coli* can also be applied to class I MHC molecules. The Examiner's opinion is respectfully transversed. The class I and class II MHC complexes are entirely different proteins with distinct protein structures. Class II MHC complex is composed of two transmembrane glycoprotein chains of α (34 kDa) and β (29 kDa) which form a heterodimer of four domains; α1/β1 form peptide binding site; α2 and β2 are Ig-like, and both chains are membrane bound. On the other hand, class I MHC is a heterodimer composed of a membrane spanning heavy α chain (43 kDa) and a non-covalently bound, non-membrane spanning β2-microglobulin polypeptide (12 kDa). The heavy chain has three extracellular domains: α1, α2 and α3, and the α1/α2 form the peptide binding site, α3 and β2-m are Ig-like. Moreover, these proteins are also different from each other in the site of peptide acquisition. While in class I MHC molecules, peptide acquisition takes place in the endoplasmatic reticulum during biosynthesis as well as on the cell surface when exposed to exogenous peptides, in class II MHC molecules, peptide acquisition takes place in the endosome or lysosome, where degraded products are encountered. In addition, in class II, the binding of peptides is mediated by H-2M (in mouse) or HLA-DM (in human). Thus, since these proteins traverse through different cellular compartments from their biosynthesis to their maturation, they exhibit unique preferences for the origin of the proteins (or peptides) that they bind for antigen presentation. For further details see "Fundamental Immunology", Paul WE (Ed.), 1999; Forth edition; Lippincott-Raven publishers, "The

function of MHC molecules", pp: 265-269.

For the above stated reasons, one of ordinary skill in the art would think it highly unlikely that methods of producing class II MHC complexes in bacteria can be applied to the production of class I MHC complex. Moreover, as is mentioned above, class I MHC complexes cannot be generated without a peptide in the MHC groove and therefore are not stable without peptide. Thus, the class I MHC protein of the present invention is produced in *E. coli* as inclusion bodies and has to be refolded *in vitro* in the presence of a peptide. On the other hand, Class II molecules, which exhibit different biochemical properties and thus are more stable, can be expressed in *E. coli* even in the absence of a peptide, as described by Altman et al.

Thus, Applicant strongly believes that one of ordinary skill in the art would not contemplate applying approaches for class II MHC generation to class I molecules since it is not apparent how use of such approaches would lead to success especially in light of the fact that class I studies concentrated their efforts on separate expression of the two MHC components in eukaryotic cells as taught by Matsumura et al.

In view of the above remarks it is respectfully submitted that claims 5-14 are now in condition for allowance. Prompt Notice of Allowance is respectfully and earnestly solicited.

Respectfully submitted,

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Encs.:
Extension fee: one month
Ljunggren HG et al., 1990; Nature (London) 346: 476-480;
Paul WE (Ed.), 1999; Fundamental Immunology, Forth edition; Lippincott-Raven publishers, pp. 273 and 265-269.